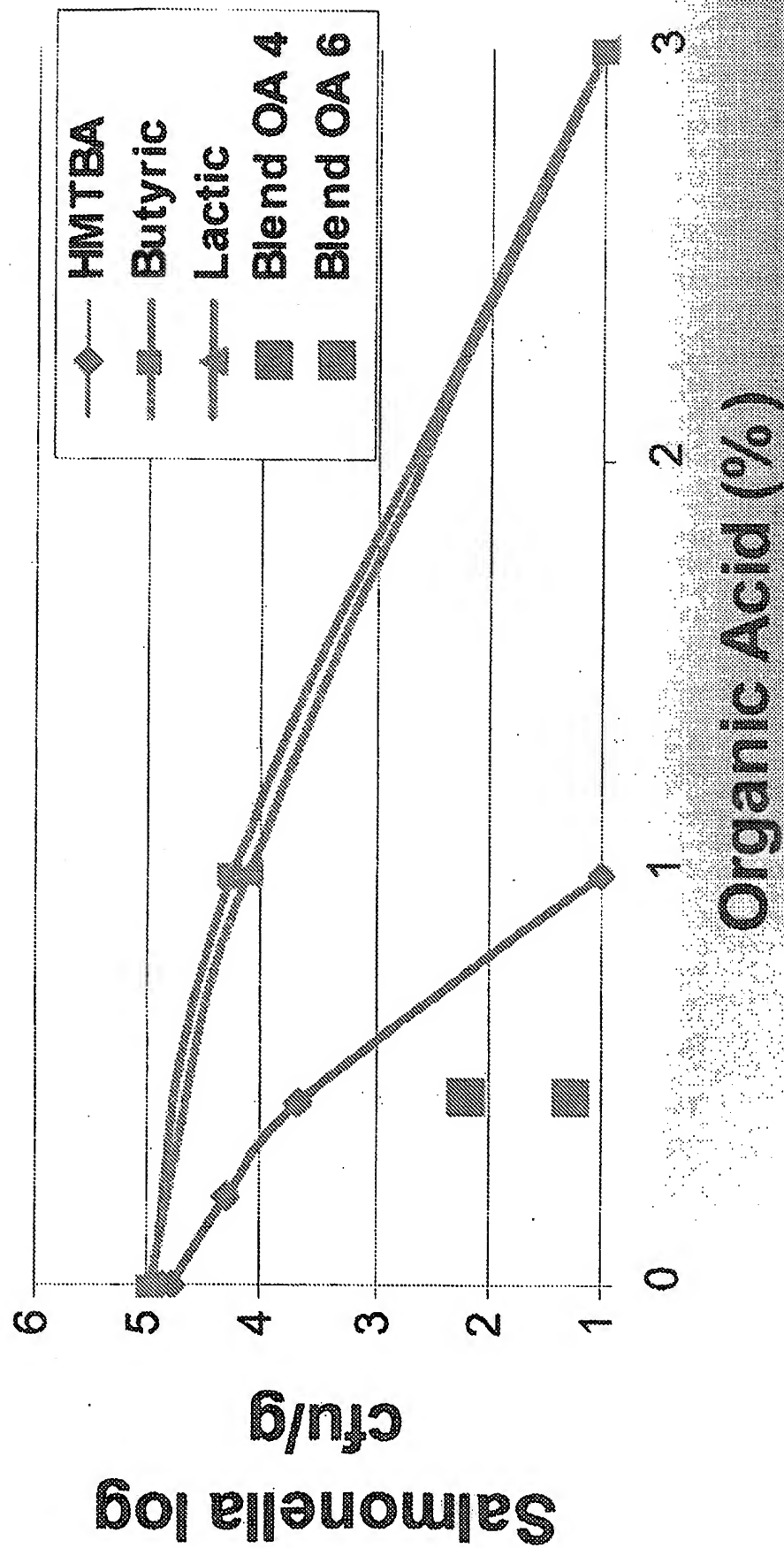
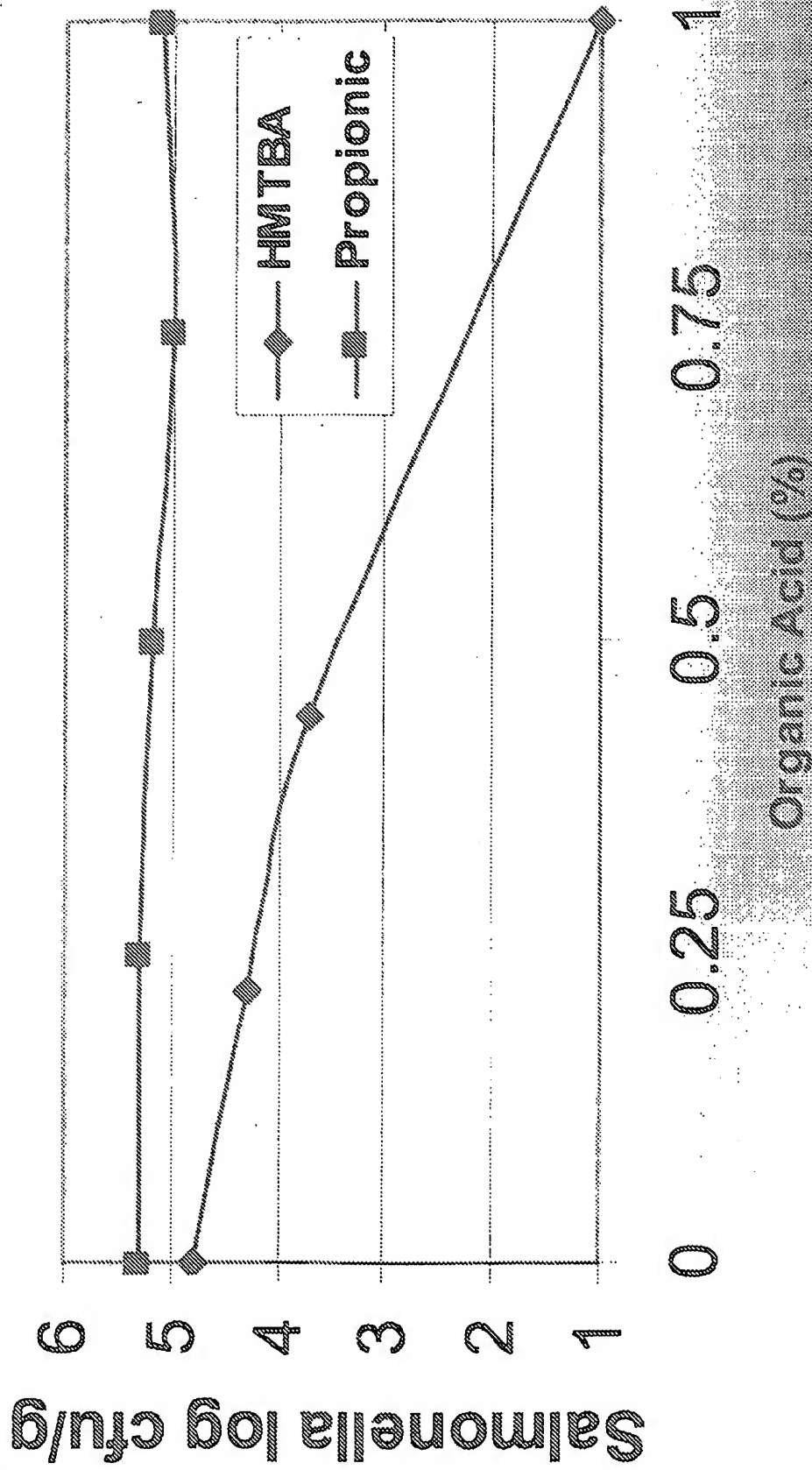


Figure 7. Increased effect of HMTBA-containing organic acid blends (Activate) against *Salmonella* (in feed for 90min, 37C, pH 4)



Comparison of HMTBA & Propionic on *Salmonella* in feed for 90min, 37°C, pH 4



UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Schasteen et al.	Art Unit	1617
Serial No.:	10/652,745	Examiner:	S. Kantamneni
Filed:	August 29, 2003	Conf. No.	1765
For:	ANTIMICROBIAL COMPOSITIONS		

DECLARATION OF CHRISTOPHER D. KNIGHT UNDER 37 C.F.R. § 1.132

I, Christopher D. Knight, declare and state as follows:

1. I have over twenty years of experience in the field of animal health and nutrition. Novus International Inc., a global leader in animal health and nutritional products, currently employs me as Vice-President for Research and Development. My employment by Novus International has been continuous for over sixteen years. Prior to my employment at Novus International Inc., I was employed by Monsanto in their Animal Sciences Division for over five years. My educational background includes a Bachelor of Science degree in Animal science awarded by Cornell University in 1975; a Master of Science degree in Monogastric Nutrition awarded by Purdue University in 1977; and a doctorate degree (i.e., Ph.D.) in Monogastric Nutrition awarded by Purdue University in 1981. I have also published over approximately thirty journal articles or posters at internationally attended meetings, and I am an inventor on three patents. Attached to this Declaration is a copy of my curricula vitae.
2. I have reviewed U.S. Patent Application Publication No. 2004/0175434 ('434 application) entitled "Antimicrobial Compositions." The '434 application has claims directed toward antimicrobial compositions that comprise several organic acid formulations developed at Novus, and presently sold under the trade name ACTIVATE®.
3. Through my position at Novus as Vice-President for Research and Development, I am familiar with and supervised portions of the research and development efforts that resulted in the discovery of several organic acid blends, which are claimed in the '434 application. The focus of this research effort was to improve the cost effectiveness of the formulations, while at the same time improving the antimicrobial activity of the blend of organic acids compared to any individual organic acid comprising the blend. The ACTIVATE® organic acid

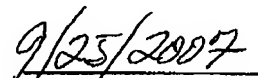
PATENT

Atty. Docket No.: 117961
Via EFS-Web

formulations (as described in various iterations of the '434 application), in my opinion, meet both of the aforementioned goals.

4. We have research data, that in my opinion, demonstrates surprising and unexpected results for organic acid formulations falling within the scope of the '434 patent claims. As an example, attached to this Declaration is a graph (identified as figure 7) that depicts a synergistic effect for two organic acid formulations of the claimed invention. With reference to the attached graph, data is depicted for the antimicrobial activity of five different organic acid compositions against *Salmonella* in feed. The five organic acid compositions include: (1) 0.45% HMTBA alone (i.e., 2-hydroxy-4-(methylthio)butanoic acid, which is a compound of Formula (I) in the '434 application); (2) 0.45% butyric acid alone; (3) 0.45% lactic acid alone; (4) blend OA 4, which is 0.15% lactic acid, 0.15% propionic acid, and 0.15% HMTBA; and (5) blend OA 6, which is 0.1% lactic acid, 0.1% butyric acid, 0.1% propionic acid, and 0.15% HMTBA. The antimicrobial experiments were conducted in accordance with Novus's standard protocol entitled "Low pH in Feed Test Procedure," a copy of which is attached to this Declaration. As depicted in the graph, the antimicrobial activity of either blend OA 4 or blend OA 6 achieved significantly higher killing of *Salmonella* at lower concentrations than could be achieved with any of the single organic acids alone.
5. I further declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.


Christopher D. Knight


Date

CURRICULUM VITAE

Christopher D. Knight, Ph.D

31 Ranch Court
St. Louis, MO 63146
(314) 567-6627 (h)
(636) 926-7401 (o)

Education

1977- 1981	Ph.D. in Monogastric Nutrition Purdue University, West. Lafayette, IN Department of Animal Science. Graduate Instructorship, 1977-1981
1975- 1977	M.S. in Monogastric Nutrition Purdue University, West. Lafayette, IN Department of Animal Science. Graduate Research Assistant
1973- 1975	B.S. Animal Sciences Cornell University, Ithaca, NY
1971- 1973	A.A.S. Science Laboratory Technology State University of New York at Cobleskill

Employment

2001- Present	Department Head, Research & Development Novus International, Inc.
1996- 2001	Director New Business Development Novus International, Inc.
1991- 1995	Manager and Director Nutrition Research Novus International, Inc
1987- 1991	Research Group Leader Monsanto Company Animal Sciences Division Porcine Somatotropin Group
1981- 1986	Research Specialist and Research Group Leader Monsanto Company Alimet Metabolism and Applications Research Group

Key Accomplishments

- Developed foundation data quantifying availability of ALIMET® Feed Supplement as a by-pass methionine source in lactating dairy cattle and methods to predict methionine deficiency using existing nutritional models. These data resolved decades of research work to attempting to commercialize this product application that had failed due to unpredictable field results. The research demonstrated Alimet to be the most cost-effective source of post-ruminal methionine activity available, resulted in a US patent and the development of a \$5M/yr business for Novus. As of 2005, a new Ruminant Business Unit of 20 employees and agents and a portfolio of 8 products (including Alimet and MHA) for the dairy industry has been formed.
- Led the development and commercialization of OASIS® Hatchling Supplement, a hydrated nutritional supplement fed to young poultry in transit or to stimulate rapid onset of ad libitum feeding after placement. This patented product developed a new market in the poultry industry based on developmental research at Novus showing the impact of early nutrition on subsequent long term performance and health. Cumulative sales of this niche product have exceeded \$4M and resulted in the development of gastrointestinal health as a core research and development competency within Novus.
- Led the technology development, regulatory approval and early commercialization of ADVENT® Coccidiosis Control, an orally applied coccidiosis vaccine based upon technology that permits the in vitro determination of oocyst viability such that a vaccine of consistent potency can be produced and marketed. This represented a new area of technology for Novus and in 2003, a jury of scientists and technology experts from Washington University and St. Louis University awarded the developers of this technology (Dr. Julia Dibner and Dr. Chris Knight) with The St. Louis Technology Award. The Advent Coccidiosis Control technology was among eight other winners from approximately 70 nominations in the St. Louis vicinity. In determining winners, the judges considered the scope, economic impact and overall significance of the new technology. Facilitated by the Academy of Science of St. Louis, the judging process also examined the level of sophistication of the entries and the innovation utilized to bring it to fruition. This technology represents a keystone of a business strategy that focuses on gastrointestinal health and drug-free poultry production.
- Established a new cost-efficient method of product development research, to insure Novus' capability to conduct scientifically and commercially relevant research across multiple species without requiring ownership or hands on care and management of research facilities. Initially divested Novus-owned animal research facilities and sought collaborative investment opportunities with scientific professionals in animal agriculture to provide capital for research facilities that would be controlled by the research partner but provide Novus with preferred status for conduct of research. To date we have formed 3 partnerships like this in the US that permits routine product development work in broilers, swine (weaning, grow-finish and lactating sows) and dairy cattle, all in commercial scale production environments. Similar agreements are

under development in Brazil (commercial scale egg layer research) and China (commercial scale swine research including wean, grow-finish and sow nutrition).

- The foundation product for Novus International is ALIMET® Feed Supplement, a source of methionine activity referred to as methionine hydroxyl analog or chemically DL-2-hydroxy-4-(methylthio) butanoic acid. Today this business represents approximately \$400M in annual revenue to Novus in a \$1B methionine market, however, in 1981 this represented about a \$20M business. In the course of my 25 year involvement with this product there has been a heated commercial controversy with respect the relative efficacy of Alimet and the competitive product DL-methionine (DLM). A close colleague (Dr. Julia Dibner) and I have had the responsibility of understanding the absorption, metabolism and utilization of Alimet, how it differs from that of DLM and the impact that the differences have on the commercial value of Alimet relative to DLM. Today based on a variety of independent and collaborative research efforts it is understood that the metabolism of Alimet is very different from DLM, that those differences result in differences in ad libitum feed intake (less than DLM at low supplementation rates, greater than DLM at the maximum response level) resulting in different dose responses for the two methionine sources. A substantial part of the controversy was based on the a priori assumption that the two products must have the same dose response since they both provide methionine. With collaboration with various statistical experts, we have been able to establish that the two products in fact have different dose responses and have described the appropriate statistical methods for comparing two products that exhibit different dose responses (Poult. Sci. 85:947-954). The controversy will continue due to commercial conditions (Alimet is less expensive to manufacture than DLM) , however over the course of 25 years Alimet has continued to grow at a 25% compounded annual growth rate with over a 50% market share in the US. The science applied to this commercial issue has laid the technical foundation that has provided Novus with the technical credibility to expand our product offerings from amino acids into nutritional organic acid blends, organic trace minerals, ingredient preservation and coccidiosis control.

ALIMET® Feed Supplement, OASIS® Hatchling Supplement and ADVENT® Coccidiosis Control are registered trademarks of Novus International, Inc., St. Louis, MO.

Personal

- Married 1982: Sandra J. Rogers (Purdue Food Science MS 1978).
- Children: Adam (19), Evan (16), Audrey (14)

Community Involvement

- Subdivision Trustee: 1987-1989: Led resolution of road and storm sewer repair dispute
- St. Peter's Episcopal Church:
 - Youth Sponsor: 1984-1988
 - Sunday School Teacher: 1992-2006 (Variety of grades and curricula)
 - Vestry: 1989-1993
 - Founding Christian Education Commission & Chair: 1989-1993
 - Confirmation Teacher: 2005-6.
 - Founding and sustaining member of Haven of Grace: Home for unwed mothers
- Hobbies
 - Cooking
 - Gardening
 - Kid's Sports

PUBLICATIONS & PROCEEDINGS

1. Dibner, J.J. and **C.D. Knight** (1984) Conversion of 2-hydroxy-4-(methylthio) butanoic acid to L-methionine in the chick: A stereospecific pathway. J. Nutr. 114:1716-1723.
2. **Knight, C.D.** and J.J. Dibner (1984) Comparative absorption of 2-hydroxy-4-(methylthio)butanoic acid and L-methionine in the broiler chick. J. Nutr. 114:2179-2186.
3. Dibner, J.J., F.J. Ivey, C.Q. Lawson and **C.D. Knight** (1986) *In vitro* methods in animal nutrition. Proceedings of the Conference European D'Aviculture 7:312-316.
4. Dibner, J.J., **C.D. Knight**, R.A. Swick and F.J. Ivey (1987) Absorption of 2-hydroxy-4-(methylthio) butanoic acid from the hindgut of the broiler chick. Poult. Sci. 67:1314-1321.
5. Dibner, J.J., **C.D. Knight**, C.Q. Lawson, R.A. Swick and F.J. Ivey (1990) Studies of the metabolism of 2-hydroxy-4-(methylthio)butanoic acid (HMB, Alimet®) in the broiler chick using *in vitro* methods. Memorias: XI Congreso de Avicultura Centroamericano y del Caribe, pp15-18.
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13. Hammond B.G., Vicini J.L., Hartnell G.F., Naylor M.W., **Knight C.D.**, Robinson E.H., Fuchs R.L., Padgett S.R. 1996. The feeding value of soybeans fed to rats, chickens, catfish and dairy cattle is not altered by genetic incorporation of glyphosate tolerance. *J Nutr.* 1996. 126(3):717-27.
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32. G. F. Yi, J. J. Dibner, C. S. Schasteen, J. Wu, K. R. Perryman, and **C. D. Knight**. 2006. Evaluation of 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) and HMTBa containing ACTIVATE® nutritional feed acid blend in different nursery pig feeding programs. J. Anim. Sci. (Submitted).

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1. Ivey, F.J., J.J. Dibner, and **C.D. Knight**, 1999. Nutrient formulation and process for enhancing the health, livability, cumulative weight gain or feed efficiency in poultry and other animals. Patent number 5,976,580.
2. Ivey, F.J., J.J. Dibner, and **C.D. Knight**, 1999. Nutrient formulation and process for feeding young poultry and other animals. Patent number 5,985,336.
3. **Knight, C.D.**, K. Koenig, L. Rode, M. Vandenberg, and M. Vázquez-Añón 2000. Process for optimizing milk production. Patent number 601,753

TITLE: Low pH in Feed Test Procedure**METHOD NO.****MATERIAL: Activate DA™****TEST: Anti-bacterial activity of organic acids measured in feed at low pH**

SCOPE: Anti-bacterial activity of organic acids is measured in feed at low pH to simulate the low pH and moisture conditions in the upper digestive tract of animal.

MATERIALS:

1. Finished feed: mash or crumble, swine or poultry
2. Fresh culture of *Salmonella* and *Escherichia coli*
4. Brilliant Green Agar or other selective media for salmonella enumeration
5. MacConkey Agar or other selective media for e. coli enumeration
6. Incubator set at 40C for the assay, and 37C for bacteria enumeration (plating)
7. Pipettes and sterile tips
8. Sterile tubes (50 ml)
9. Hydrochloric acid

SAFETY CONSIDERATIONS:

1. Mouth pipetting is not allowed, automatic pipettes or pipette bulbs must be used.
2. Use appropriate gloves where necessary.
3. Dispose of all hazardous waste properly. Autoclave all wastes containing salmonella or e. coli.

PROCEDURE:**Prepare fresh cultures of salmonella and e. coli:**

1. Grow a fresh culture of salmonella or e. coli overnight at 37C in Tryptic Soy Broth (or appropriate media for the particular strain of bacteria)
2. Determine the counts by direct plating
3. Keep the culture at 4C until use. Prepare fresh cultures every 2 weeks.

Determine the amount of HCL needed to bring the feed to pH 4.0

1. Prepare 150mM HCL solution from concentrated HCl (12.1N HCl),
2. Weight out 5g of mash or crumbled feed in 50ml tubes,
3. Add 150mM HCl and DI H2O at different proportions (see the table below) to achieve a total volume of 15 ml,

150mM HCl	7.25 ml	7.50ml	7.75 ml	8 ml	8.25ml
DI H2O	7.75 ml	7.50ml	7.25 ml	7 ml	6.75ml
Total volume	15 ml	15ml	15 ml	15 ml	15 ml

4. Vortex the samples for ~1 min, keep at 40C for ~20min (preferable with mixing) for the pH to equilibrate,

5. Adjust the ratio between HCl and H₂O until the pH of the feed is at ~ 4.0 (A range of 3.8 to 4.0 is acceptable).

Set up the following treatments (in 50 ml sterile tubes):

	Treatments	Dose	Reps.	Feed	Inoculant (cfu/g of feed)
1	control		2-3	5 gram	40,000
2	Activate DA	0.3%	2-3	5 gram	40,000
3	Activate DA	0.5%	2-3	5 gram	40,000

1. Weigh out 5g of finished feed in a sterile 50 ml centrifuge tube.
2. Add Activate DA to treatments 2 and 3 (15mg in the 0.3% treatment, and 25mg in the 0.5% treatment).
3. Add HCl and DI H₂O to bring the pH to 4.0 (pre-determined for each feed, see the procedures above),
4. Inoculate with Salmonella or E. coli to give a final concentration of 40,000 cfu per ml of sample (40,000 cfu/ml x 15 ml = 600,000 cfu/tube).
5. Incubate the samples for 90 minutes in a 40C incubator (preferably with mixing on an end to end rotator, but not required).
6. At the end of 90 minutes incubation, prepare 1:10 dilution of sample in sterile H₂O (1ml sample and 9 ml H₂O)
7. Plate the following samples on Brilliant Green agar (*salmonella*) and MacConkey agar (*E. coli*) and incubate plates at 37C overnight.
 - 100ul of 1:10 dilution from step 6
 - 100ul of undiluted sample
8. Count colonies the next day, determine cfu/ml sample, and compare with control.

ANALYTICAL TIME:

REFERENCE:

ATTACHMENTS : None

DOCUMENT CONTROL DATES :

Issue & Effective Date:

Prepared/Revised by: Date:

Approved by: Date:

Effect of Alimet and OA on E. Coli In swine diet at pH 4.0 (with pre-equilibrated pH)

Feed: Ian's diet A, no DLM or alimet
 1g feed +2 ml 150mM HCl + 1 ml DH₂O, 30mins at 37C before the addition of treatment
 E. coli added at 400,000 cfu/g
 experiment repeated 2 times, duplicate samples in each run

Treatments:

- 1 control
- 2 0.15% alimet
- 3 0.15% butyric/0.15% lactic/ 0.15% alimet
- 4 0.15% lactic/ 0.15% propionic/ 0.15% alimet
- 5 0.15% butyric/0.15% propionic/ 0.15% alimet
- 6 0.1% lactic/0.1% butyric/0.1% propionic/0.15% alimet
- 7 0.3% lactic/0.15% alimet
- 8 0.3% fumaric/0.15% alimet
- 9 0.3% propionic/0.15% alimet
- 10 control

Treatment	E. coli recovered	ave	log cfu/g	diff.	exp #1	ave	log cfu/g	diff.	ave (1+2)	ave reduction
1	64000	175000	119500	5.1	632000	436000	534000	5.7	5.3	
2	700	6700	3700	3.6	400000	234000	317000	5.5	0.2	
3	7000	112000	59500	4.8	284000	274000	279000	5.4	0.3	5.1
4	6400	104000	55200	4.7	85000	82000	83500	4.9	0.8	4.8
5	57000	25000	41000	4.6	73000	226000	149500	5.2	0.5	4.9
6	38000	7000	22500	4.4	228000	250000	239000	5.4	0.3	4.9
7	21000	2000	11500	4.1	104000	168000	136000	5.1	0.6	4.6
8	71000	28000	49500	4.7	62000	1000	31500	4.5	1.2	4.6
9										0.7
10	69000	42000	55500	4.7	420000	316000	368000	5.8		

